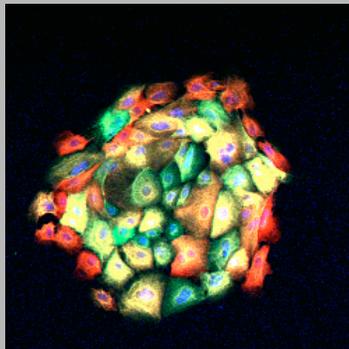


NCAB

Discussion of Cancer Stem Cell Theory
September 15, 2009

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Cancer Stem Cell Theory

- Measures the heterogeneity of cellular populations within tumors
 - Tumor initiation
 - Self-renewal
 - Marker expression
- Tumor initiating populations
 - Express markers of progenitors
 - Produce heterogeneous tumors

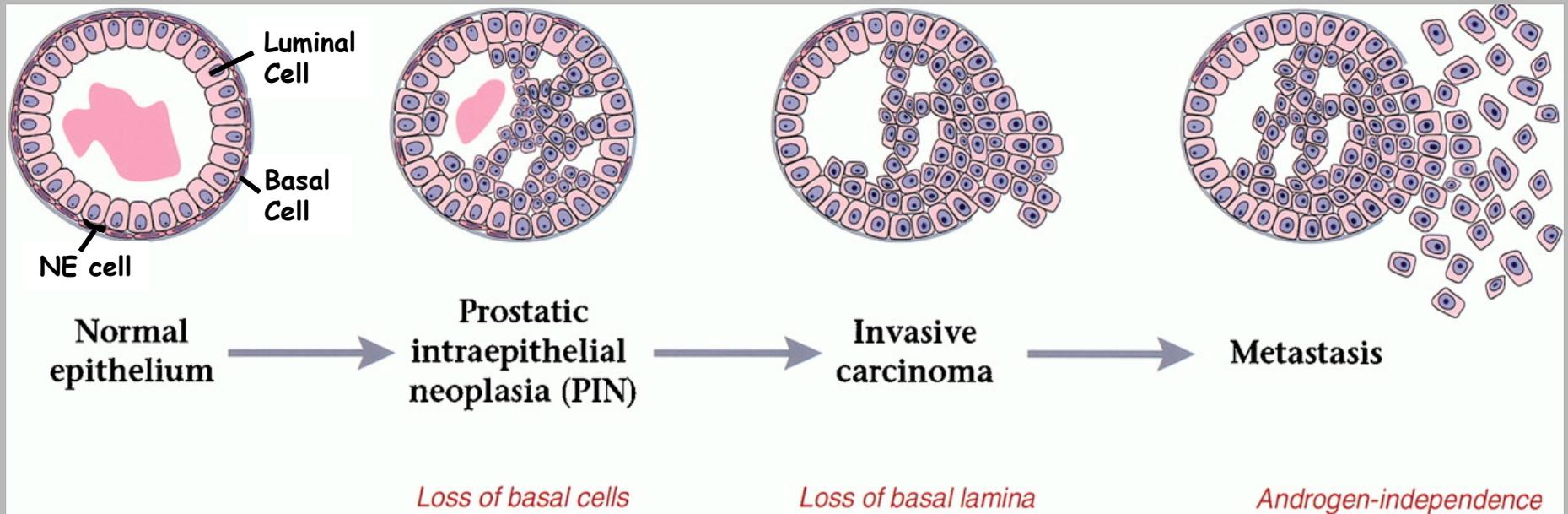
Clarifications re CSC Theory

- Does not make assumptions about the frequency of tumor initiating cells
- Does not make assumptions about the cell of origin
- Does not discount the possibility of plasticity: non-CSC may convert to CSC's

The Value of Investigating Tumor Heterogeneity

- Defining the cell of origin will lead to better early detection markers
- Treatments must target all populations
- Cancer stem cells and metastasis initiating cells share several properties

Prostate Cancer Progression



Luminal Cells: CK8+

Basal Cells: CK5+, p63

Neuroendocrine Cells: synaptophysin+, β -3 tubulin

Prostate Stem Cell Properties

- Resistant to castration
- Cofractionate with basal cells using surface markers
- Give rise to luminal, basal, and NE cells

Properties of PC Metastasis

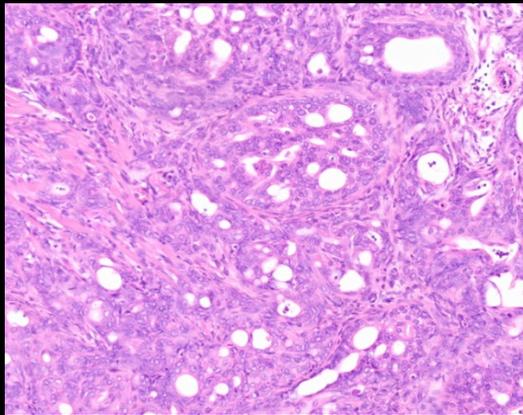
- Poorly differentiated CK8+ carcinomas
- Heterogeneity among metastases within a single patient
 - Variable neuroendocrine differentiation
 - Variable androgen receptor expression
- A large percentage of castrate-resistant prostate cancers express mutated AR, suggesting evolution from an AR+ cell

Questions Being Addressed

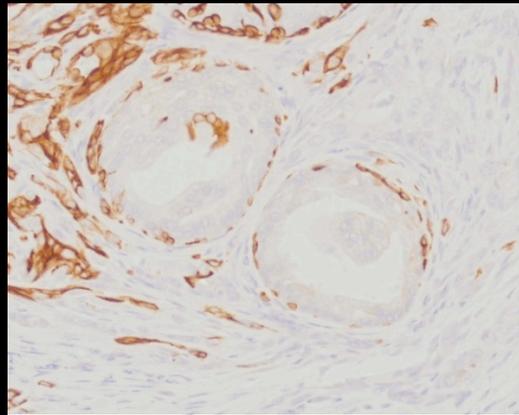
- Mechanistic effect of specific common gene mutations on prostate progenitor populations
- Cellular origins of castrate-resistant PC and physiological role of AR
- Does castrate-resistant PC arise from an androgen-independent progenitor cell ?

Modeling PC in the mouse: $(PbCre^+)$ $PTEN^{fl/fl}$, $P53^{fl/fl}$, Luc^+

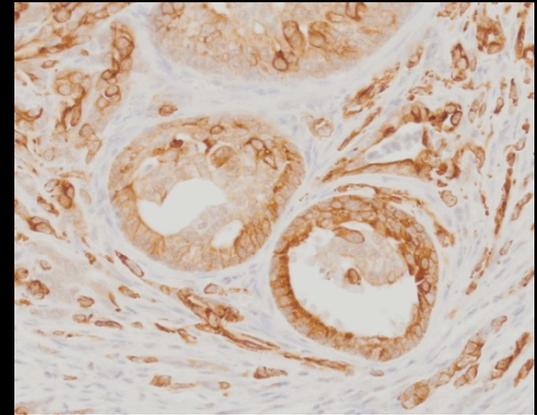
- The PTEN pathway is frequently altered in human PC
- Development of invasive and disseminated adenocarcinoma, but not clinically-apparent metastatic tumors
- Death from urinary outflow obstruction at ~ 6 mos.
- Proliferation of multiple epithelial cell types (basal, intermediate, and luminal)



H&E

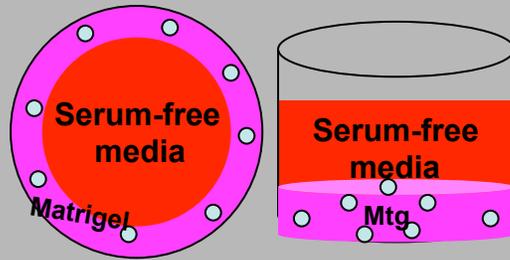


CK5

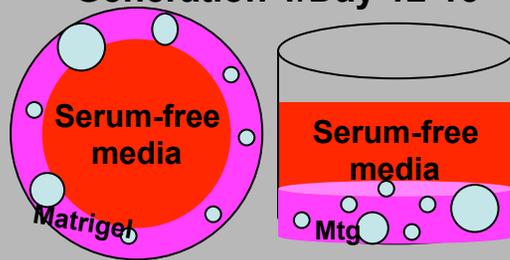


CK8

Sphere-forming assay (3-D)

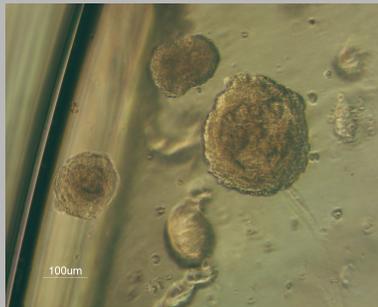


Generation 1/Day 0

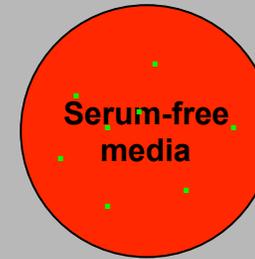


Generation 1/Day 12-15

- Single cell
- Sphere

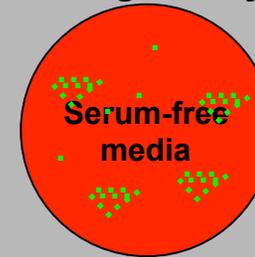


Colony-forming assay (2-D)

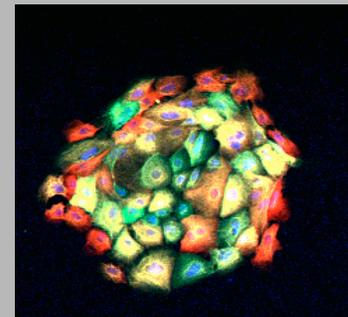


Passage 0/Day 0

Passage 0/Day 5-8

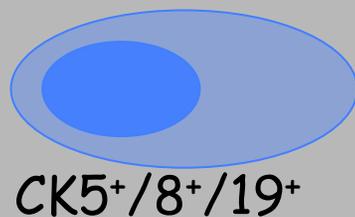


- Single cell
- Colony

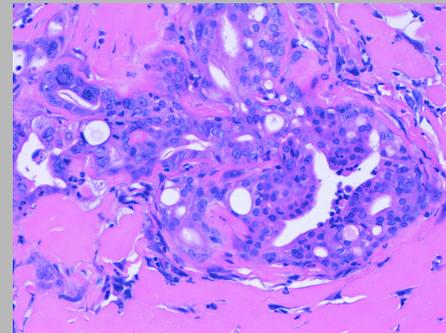


Prostate progenitors are tumor initiating cells

1. Cell clones established from tumors and expressing markers of progenitor cells give rise to adenocarcinoma



Orthotopic
injection →

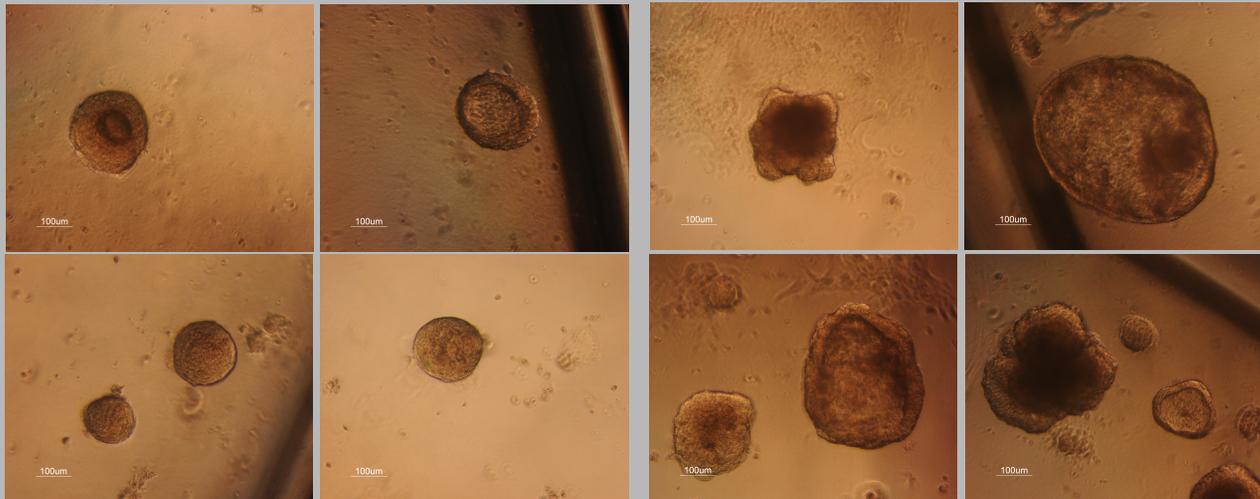


2. Single cell suspensions made from protospheres give rise to prostate carcinoma

Protosphere Morphologies

WT G1/D12

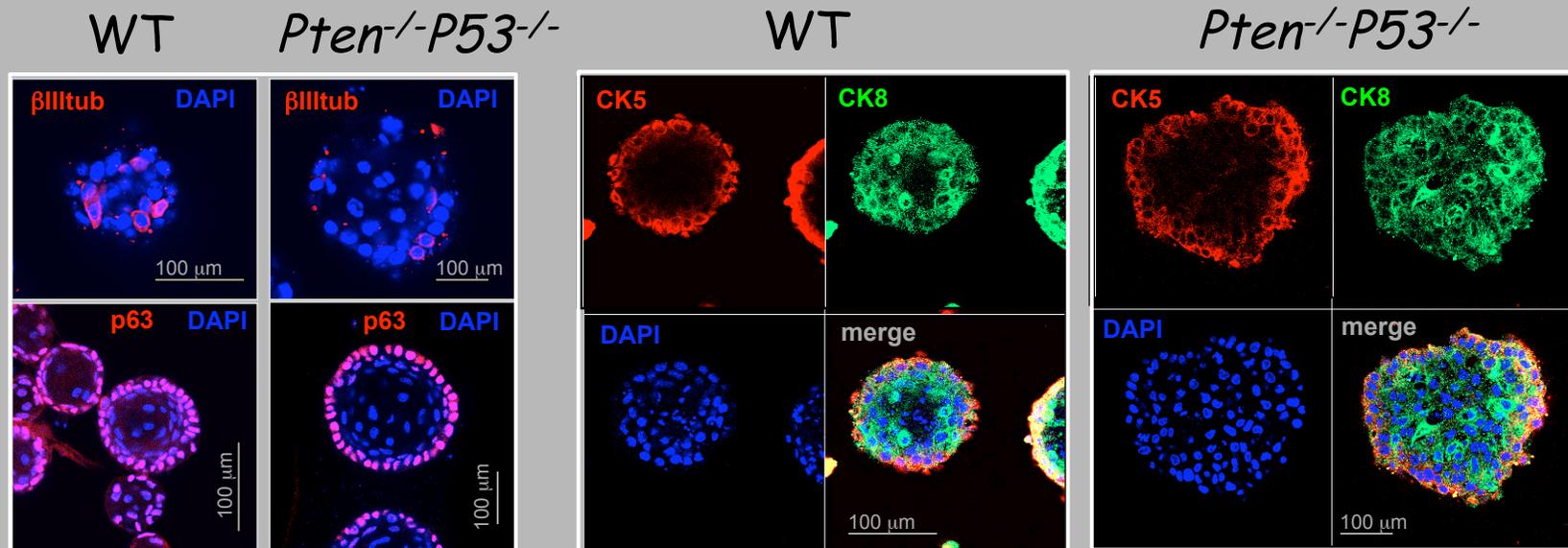
Pten^{-/-}P53^{-/-} G1/D12



Pten^{-/-}P53^{-/-} protospheres relative to wt are

- 3X larger in diameter
- Contain 50% more cells

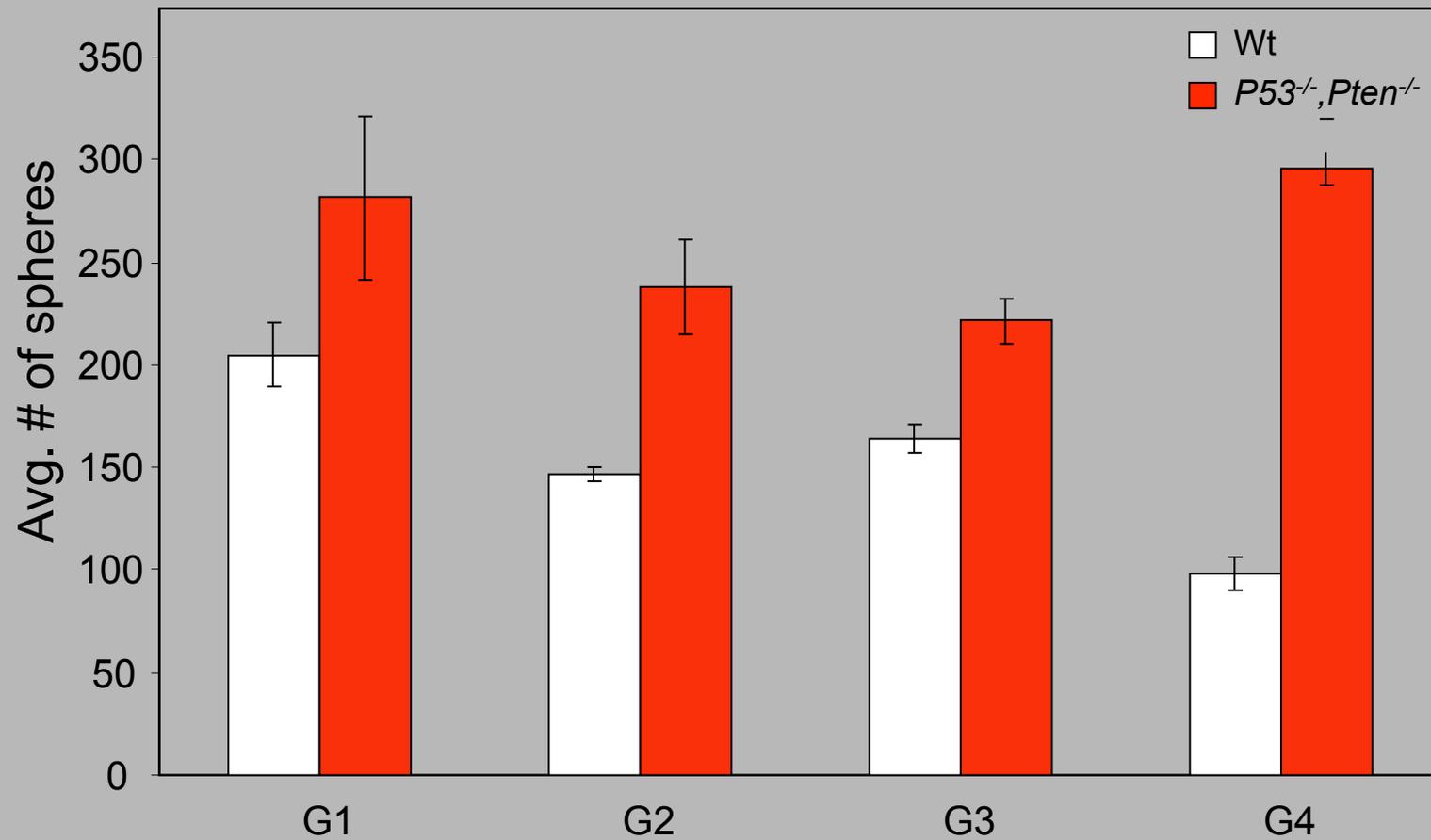
Differentiation Potential in Transformed spheres



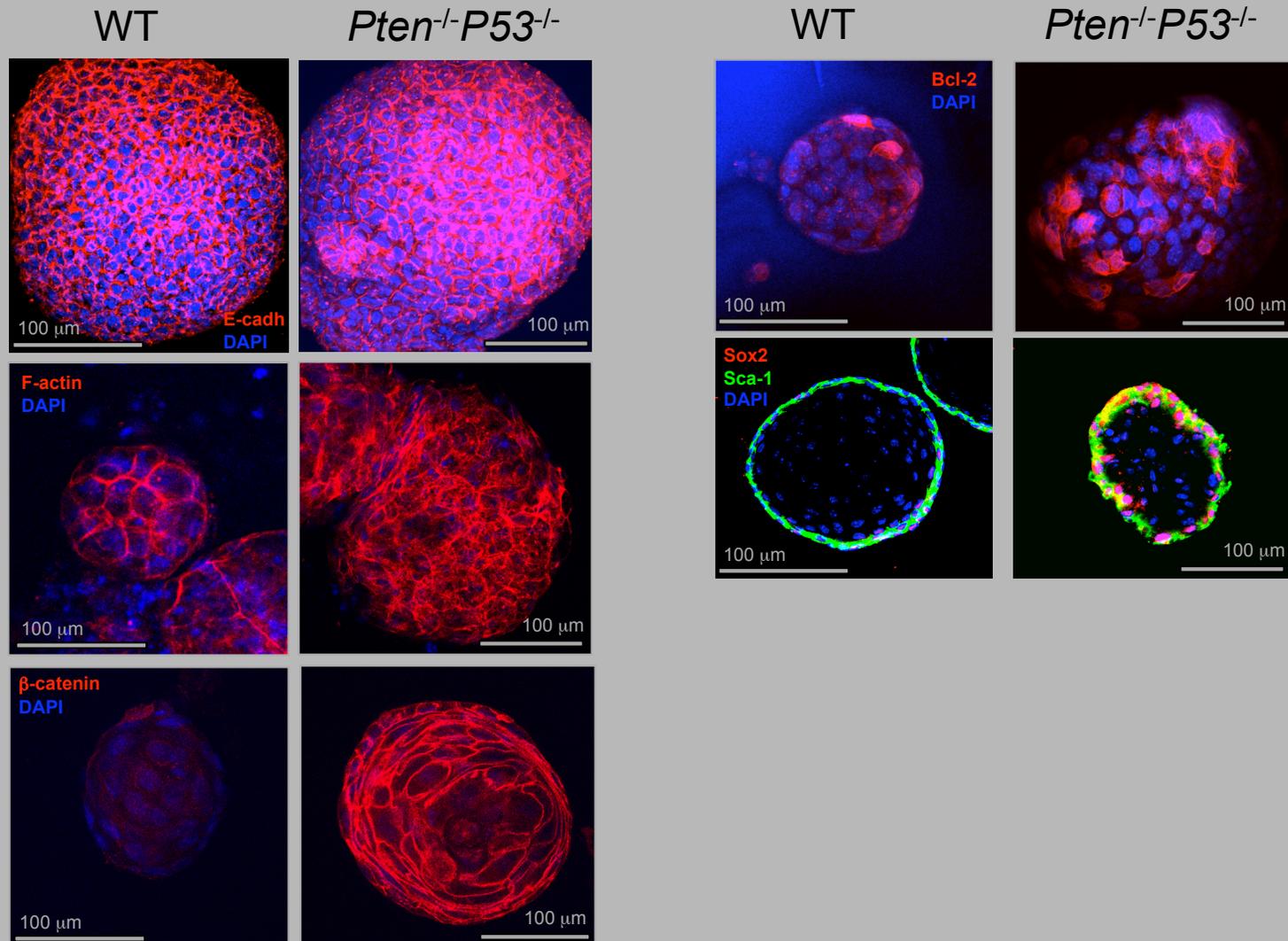
- Sphere-forming cells are rare (~1%)
- Spheres contain multipotent progenitors that produce basal, intermediate (CK5⁺/CK8⁺), and neuroendocrine cells
- Spheres have a defined architecture
- Basal cells form the outermost layer
- *Pten*^{-/-}, *P53*^{-/-} progenitors produce more CK8⁺ cells

Transformed Progenitors Show Increased Self-Renewal

Sphere formation assay

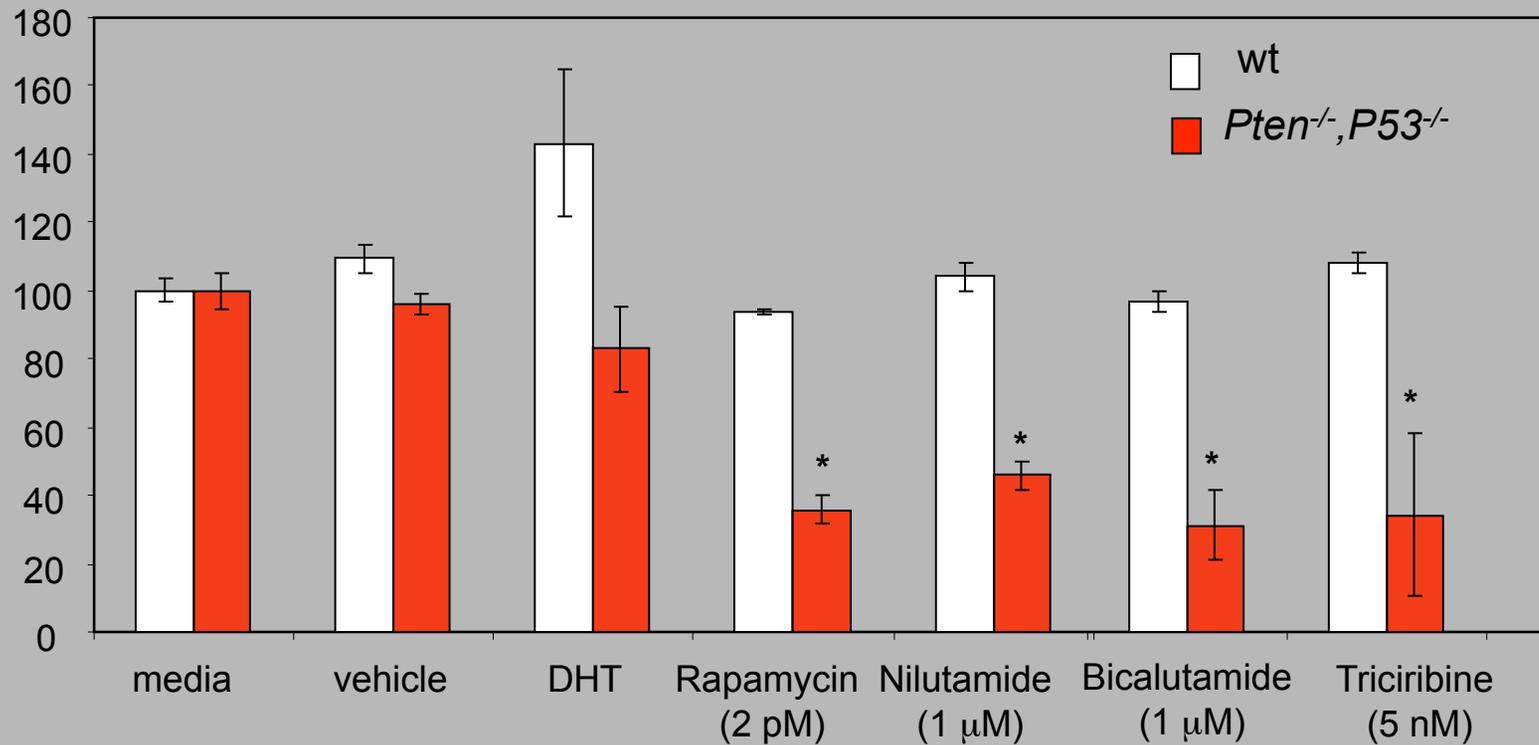


In situ Assays of Differentiation and Signal Transduction Markers



Transformed Progenitors Are Differentially Inhibited by Drugs

Colony formation (% of ctrl)



Conclusions

- PTEN^{-/-};P53^{-/-} prostate progenitors demonstrate perturbations of self renewal and differentiation
- These progenitors express altered drug sensitivity- i.e. AKT "addiction" and acquired AR dependence

Implications

- Establishing the relationship of specific gene mutations to CSC function is important for improved mechanistic understanding of cancer progression and treatment
- Therapeutic screening methodologies that target unique CSC signaling properties should be developed



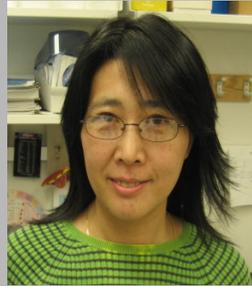
Philip Martin
Rachel Pierce



Wassim Abou-Kheir



Paul Hynes



Ivy Yin



Orla Casey



Luhua Zhang



Yvona Ward



Ross Lake